PHOTOBIOLOGY/PHOTOMEDICINE

Milestones in Photoimmunology

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doi:10.1038/skinbio.2013.177

Photoimmunology started almost 40 years ago with the discovery of a link between UVR and immunosuppression during experimental photocarcinogenesis. Cutaneous UVR-induced tumors appeared to be highly antigenic, as they were rejected upon inoculation into syngeneic mice (Kripke, 1986). Recipients treated with immunosuppressive drugs did not reject the transplanted tumors, similar to recipients who had received low doses of UVR instead of pharmacological immunosuppression, indicating that UVR suppresses cellular immunity. The most effective UVR spectrum to alter an immune response turned out to be the mid-wave range (UVB, 290–320 nm). Thus, most of the photoimmunologic studies were conducted with UVB, although UVA (320–400 nm) certainly has an effect on immune reactions as well.

Besides the development of skin tumors, UVR was found to inhibit contact hypersensitivity (Toews *et al.*, 1980). As mice that received the contact allergen through UVR-exposed skin could not be resensitized against the same antigen a few weeks later, it was concluded that UVR induces long-term suppression. This appeared to be antigen specific, as no other immune responses were altered. As UVR exposure and sensitization affect the same skin area, this model is also referred to as *local immunosuppression*. Later studies showed that UVR, although at higher doses, can also affect the immune system in a systemic manner via the release of soluble mediators (IL-10, tumor necrosis factor) from the skin (Ullrich and Schmitt, 2000). Whether the alarmin (endogenous mediators released upon cellular injury) IL-33 contributes to UVR-induced immunosuppression awaits further investigation, although the fact that neutralization of IL-33 blocks UVR-induced immunosuppression is quite convincing (Byrne *et al.*, 2011).

THE CHANGING ROLE OF LANGERHANS CELLS

Soon thereafter, it was shown that the antigen-specific immunotolerance induced by UVR is attributable to the occurrence of T cells with suppressive activities (Elmets *et al.*, 1983), at that time called ''suppressor T cells'' and these days renamed ''regulatory T cells'' (Tregs) (Schwarz, 2008; Loser and Beissert, 2012). The same low doses of UVR that suppressed sensitization depleted the Langerhans cells (LCs) from the epidermis. Initially, it was assumed that LCs are killed by UVR, but later it turned out that they leave the epidermis. As in those days LCs were regarded as the most important antigen-presenting cells in the skin (Romani *et al.*, 2012), the failure to induce sensitization through UVR-exposed skin was associated with depletion of LCs from the epidermis.

Nowadays, the functional role of LCs has been redefined. It became clear that LCs are not the primary antigen-presenting cells in the skin, but that this job is mostly done by dermal dendritic cells (Merad *et al.*, 2008). In contrast, LCs appear to be more involved in downregulating than inducing immune responses, as recently demonstrated in diphtheria toxin receptor knock-in mice in which LCs can be depleted via injection of diphtheria toxin (Bennett and Clausen, 2007). Similarly, it was shown that LCs are crucially involved in UVR-induced immunosuppression, as inhibition of the induction of contact hypersensitivity (CHS) and the generation of Tregs were no longer observed upon UVR in LC-depleted mice (Schwarz *et al.*, 2010). This is in accordance with the previous finding that the appearance of UVR-damaged LCs in the regional lymph nodes was required for the induction of Tregs (Schwarz *et al.*, 2005). This implied that damaged but still alive LCs present the antigen in a nonprofessional manner and thus do not induce T-effector cells, but Tregs. However, additional mechanisms seem to be involved, as it was observed that UVR-induced upregulation of CD254 (RANKL) on keratinocytes activated RANK-stimulated LCs to induce Tregs (Loser *et al.*, 2006).

UVR-INDUCED TREGS

UVR-induced Tregs appear to belong to the $CD4^+CD25^+$ subtype, express CTLA4, and release IL-10 (Schwarz, 2008). They express the lymph node–homing receptor CD62L, migrate into the lymph nodes, and thus primarily inhibit sensitization. However, when present in the periphery, they also suppress the elicitiation of CHS. The pattern of tissue-homing receptors can be altered by tissue-specific antigen-presenting cells (Schwarz *et al.*, 2011). Upon contact with LCs, UVR-Tregs downregulate CD62L and express skin-homing receptors. These cells mediate their effect by migrating into the skin and inhibit the elicitation. Hence, UVR-Tregs can suppress both the induction and the elicitation of CHS, provided they are at the appropriate site.

INVOLVEMENT OF CELLS BEYOND T CELLS

When studying T-cell subsets involved in UVR-induced immunosuppression, it became evident that *natural killer T (NKT) cells* are involved in the suppression of tumor immune responses by UVR (Moodycliffe *et al.*, 2000). NKT cells that express intermediate amounts of T-cell receptor molecules and coexpress surface antigens normally found on NK cells (NK1.1, DX5, and Ly49a) may have a critical role in regulating the growth of UVR-induced skin cancers.

Mast cells were previously largely ignored in photoimmunology. However, the prevalence of murine dermal mast cells correlated directly with their susceptibility to UVB-induced systemic immunosuppression, as demonstrated in mast cell–depleted ($^{Wf/Wf}$) mice (Hart *et al.*, 1998). In addition, mast cells can be a source for IL-10, which mediates tolerance in UVR-induced immunosuppression (Alard *et al.*, 2001). However, the role of mast cells in regulating CHS requires further elucidation, as depletion of mast cells by using the diphtheria toxin receptor knock-in method was not associated with an enhanced CHS response, but with a *decreased* CHS response (Dudeck *et al.*, 2011).

Another quite neglected cell deserving more attention in photoimmunology is the B cell. They appear in increased numbers in the draining lymph nodes upon UVR (Byrne and Halliday, 2005) and reveal an activated phenotype (MHCII, B220, IL-10). These cells exert suppressive activity, and thus were suggested to be called UVR-activated regulatory B cells. Studies utilizing inhibitors revealed that serotonin and platelet-activating factor are crucially involved in the activation of this possibly new B-cell subtype (Matsumura *et al.*, 2006).

MOLECULAR EVENTS IN PHOTOIMMUNOSUPPRESSION

A major molecular event in UVR-mediated immunosuppression is the induction of DNA damage. Reduction of DNA damage via exogenous DNA repair enzymes prevented the suppression of the immune system by UVR, indicating the essential role of DNA damage in signal transduction (Kripke *et al.*,1992). Similar observations were made when injecting cytokines, such as IL-12, IL-18, and IL-23, which induce DNA repair (Schwarz and Schwarz 2009). Recently, it was shown that UVR-damaged keratinocytes release noncoding RNA, which binds via Toll-like receptor-3 (Bernard *et al.*, 2012). Toll-like receptor-3 ligation on keratinocytes induces the expression of tumor necrosis factor-α and IL-6. This pathway appears to be relevant also for UVR-induced immunosuppression, as TLR3 −/− mice were found to be resistant to the immunosuppressive effects of UVR in the CHS model.

Beyond DNA, additional chromophores for UVR-induced immunosuppression were identified. Trans-urocanic acid (UCA) is a histidine-derived molecule present in the stratum corneum. UVR isomerizes *trans*-UCA to *cis*-UCA (Mohammad *et al.*, 1999). Removal of the stratum corneum by tape stripping prevented UVR-induced inhibition of CHS, suggesting that a relevant chromophore is removed (DeFabo and Noonan, 1983). Action spectra identified *cis*-UCA as the hottest candidate. Accordingly, injection of *cis*-UCA inhibited CHS. However, the mechanisms involved in UVR- and *cis*-UCA-mediated immunosuppression do not appear to be exactly the same. For example, anti-IL-10 antibodies blocked the induction of UVR-induced tolerance completely but that of *cis*-UCA-induced tolerance only partially (Niizeki and Streilein 1997).

PHOTOIMMUNOLOGIC FINDINGS IN HUMANS

UVR-induced immunosuppression in humans may represent a risk factor for skin cancer, as the incidence of UVR-induced suppression of CHS is significantly higher in skin cancer patients. A strong association of UVR-susceptible and UVR-resistant phenotypes in humans with single-nucleotide polymorphisms in the tumor necrosis factor region was found, suggesting this region to contain genes that determine the outcome of an UVR response (Yoshikawa *et al.*, 1990).

Human volunteers developed tolerance when the hapten was initially painted onto UVR-treated skin (Cooper *et al.*, 1992). UVR not only depleted LCs but also induced $CD11b^+$ macrophages, which released IL-10 (Kang *et al.*, 1994). The development of tolerance versus suppressed CHS appears to correlate with the timing of antigen application after UVR exposure. Application immediately after UVR exposure resulted in inhibition of CHS but failed to induce tolerance (Hammerberg *et al.*, 1994). The latter was only observed upon antigen application 72 hours after UVR, when LCs were depleted and Ia^+CD11b^{bright} macrophages had immigrated into the epidermis. CD11b binds the fragment of the complement component 3, iC3b, which appears to be critically involved, as C3-deficient mice were resistant to UVR-induced tolerance (Hammerberg *et al.*, 1998).

CONSEQUENCES OF UVR-INDUCED IMMUNOSUPPRESSION

The characteristic features of UVR-induced immunosuppression are its induction by low/physiologic doses, its antigen specificity, and its influence primarily on T cell–mediated immune reactions (Schwarz, 2010). The *in vivo* relevance and the biological implications of UVR-induced immunosuppression have long been, and partially remain, unclear. It is at least partially responsible for the effects of phototherapy (Weichenthal and Schwarz, 2005). UVR-induced immunosuppression certainly is involved in photocarcinogenesis. Chronically immunosuppressed persons reveal a remarkably enhanced risk for skin cancer, which correlates with cumulative UVR exposure (Euvrard *et al.*, 2003).

In addition, it was assumed that UVR-induced immunosuppression supports the exacerbation of skin infections (Chapman *et al.*, 1995). In fact, experimental studies demonstrated the suppression of T-cell reactions against microbial antigens. However, the clinical experience differs. Except herpes simplex, the risk for infections, in particular bacterial infections, after

UVR exposure is low. Atopic dermatitis is frequently superinfected with *Staphylococcus aureus,* but can be improved by UVR even without antiseptic or antibiotic measures. Hence, the question is obvious: how can an immunosuppressive regimen such as UVR improve superinfected dermatoses without worsening the infection?

The classical adaptive immune response, which involves antigen-presenting cells, T cells, and B cells, is by far less important for cutaneous antibacterial defense than the evolutionarily much older innate immune system (Medzhitov and Janeway, 2000). The latter reacts nonspecifically, but in turn is much faster and more effective. It involves neutrophils, NK cells, complement, and also antimicrobial peptides (AMPs), small molecules with effective antimicrobial activity. AMPs are produced in the skin, in particular β-defensins, psoriasin, and RNase7 (Harder and Schröder, 2005). It was recently described that UVR induces AMPs (Gläser *et al.*, 2009). As UVR disturbs the epidermal barrier (Jiang *et al.*, 2006), it appears logical that AMPs are induced upon UVR exposure as a counter-regulatory phenomenon, preventing bacterial invasion. As AMPs are induced by UVR doses in the same physiologic range as that which suppresses the adaptive immune response, it is fair to speculate that the downregulation of adaptive immunity in the skin may be beneficial as well.

The skin is an organ close to (auto)immunity. One of the best routes to immunize is via the skin; many autoimmune diseases affect the skin. The majority of these reactions are T cell-driven (Robert and Kupper, 1999). This gives rise to the hypotheses that T cells in the skin may not always be beneficial, but more often harmful. Thus, it is fair to speculate that a certain level of constant immunosuppression by daily solar exposure may prevent the induction of such adverse immune responses, but this must be confirmed by future studies.

CONCLUSION

In summary, the view of photoimmunology has changed over the past several years (Ullrich and Byrne, 2012). The mechanisms involved are much more complex than initially thought. Previously, activation of the adaptive immune system was always associated with protection. In turn, suppression was regarded as detrimental. Nowadays, we assume that a fine-tuned balance is optimal. Thus, suppression may be as relevant as induction, and replacing the negatively perceived term ''suppression'' with ''regulation'' may be appropriate. The essential challenge of regulation is the toning down of overshooting immune responses that cause damage. Natural sun exposure may have a role in this scenario. Thus, UVR-induced immunosuppression may be judged from a different angle. Low/physiologic doses of UVR inhibit the adaptive immune system but induce parts of the innate immune system. This may be beneficial in protecting from microbial attacks, but it also tones down allergic and autoimmune reactions. This is in line with the fact that ambient solar exposure is crucial and physiologic. It will be one of the major future challenges to define the optimal UVR exposure for each individual, as excessive exposure is one of the major environmental threats for human health; this includes artificial UVR exposure as well.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENTS

TS is supported by the Deutsche Forschungsgemeinschaft (SCHW625/4-2).

TO CITE THIS ARTICLE

Schwarz T, Beissert S (2013) Milestones in Photoimmunology. *J Invest Dermatol* 133: E7–E10.

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